

# Lunar cycles of reproduction in the clown anemonefish *Amphiprion percula*: individual-level strategies and population-level patterns

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**ABSTRACT:** Lunar or semi-lunar cycles of reproduction are a widespread phenomenon in marine organisms. It is common practice to infer the adaptive value of these reproductive cycles based on the population-level pattern. This practice may be flawed if there are multiple types of individuals within the population employing different reproductive cycles. Such phenotypic diversity can be thought of in terms of alternative, mixed or conditional strategies. Here, we capitalized on a long-term field study and a carefully controlled laboratory experiment of individually identifiable clown anemonefish *Amphiprion percula* to investigate the individual-level strategies underlying population-level patterns of reproduction. The field study revealed that *A. percula* exhibit a lunar cycle of reproduction at the population level, and that there is naturally occurring variation among and within individuals in the number of times they reproduce per month. The laboratory experiment revealed that the number of times individuals reproduce per month is dependent on their food availability. Individuals employed a conditional strategy with 3 tactics: reproduce 1, 2 or 3 times  $\text{mo}^{-1}$ , depending on food availability. Breaking down the population-level pattern by reproductive tactic, we showed that each reproductive tactic has its own non-random lunar, semi-lunar ( $\frac{1}{2}$  lunar) or trient-lunar ( $\frac{1}{3}$  lunar) cycle of reproduction. We conclude that the adaptive value of *A. percula* reproductive cycles, and likely the reproductive cycles of many other marine organisms, should not be inferred from the population-level pattern. Instead, the adaptive value of lunar, semi-lunar and trient-lunar cycles should be investigated for the individuals that express them.

**KEY WORDS:** Reproductive cycles · Spawning patterns · Phenotypic plasticity · Animal behavior · Adaptive value · Clownfish · Coral reef fish · Marine ecology

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## INTRODUCTION

Many marine taxa exhibit some type of lunar cycle of reproduction at a population scale (Korringa 1947). On the Great Barrier Reef, there is a well-known mass spawning of corals that occurs between the full and last quarter moons in October and November (Harrison et al. 1984, Babcock et al. 1986). A variety of other macroinvertebrates also breed on lunar or semi-lunar cycles (Christy 1978, Mercier & Hamel 2010) as do many tropical and temperate fishes (Allen 1972, Keenleyside 1972, Johannes 1978, Lobel 1978, Ross 1978, Pressley 1980, Doherty 1983, Conover &

Kynard 1984, Foster 1987, Danilowicz 1995, Gladstone 2007). Based on these population-level patterns, a suite of hypotheses for the adaptive value of breeding at particular times during the lunar cycle and/or the intrinsic benefits of synchronizing with other members of the population has been developed. These hypotheses are particularly well-developed in the coral reef fish literature, with each hypothesis focusing on a different part of the life cycle: adult biology; egg biology; larval biology and/or settler biology (Robertson et al. 1990, Robertson 1991).

First, considering the adult biology hypothesis, if adult-food availability varies on a lunar cycle, then

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selection may favor individuals that feed when food is most available and breed at other times (Allen 1972, Gladstone 2007). Second, considering the egg biology hypotheses, if males can more effectively defend against egg predators when there is more light, then selection may favor individuals that care for their eggs around the full moon (Allen 1972). Third, considering the larval biology hypothesis, if successful larval dispersal is facilitated by tidal currents, then selection may favor individuals whose eggs hatch on particular tides (Christy 1978, Johannes 1978, Ross 1978, Foster 1987). Turning to consider the settler biology hypothesis, if the quantity or quality of settlement habitat varies with the lunar cycle, then selection may favor individuals whose larvae settle at a particular time (Christy 1978). Finally, considering the intrinsic benefits of synchronization, if high densities of breeding adults, eggs, larvae and/or settlers can swamp predators, then selection may favor individuals that synchronize their breeding on any reliable cue (Keenleyside 1972, Reaka 1976, Lobel 1978, Harrison et al. 1984, Babcock et al. 1986, Foster 1987).

One hidden assumption when inferring the adaptive value of the reproductive cycle based on population-level patterns is that there is only one reproductive-cycle phenotype in the population, and that the average timing of the population is that favored by natural selection. This assumption will be violated if there are multiple reproductive-cycle phenotypes, e.g. if some individuals reproduce once per lunar month while others reproduce twice per lunar month. In such cases, the population-level pattern will be an emergent property, resulting from pooling multiple types of individuals, and inferring the adaptive value of reproductive timing based on the population-level pattern would be a flawed practice. In such cases, it will be appropriate to investigate the adaptive value of the lunar cycle for those individuals exhibiting a lunar cycle and the adaptive value of the semi-lunar cycle for those individuals exhibiting a semi-lunar cycle (Gross 1996, Davies et al. 2012). This means that determining what individuals are doing, and how this contributes to the population-level pattern, is a critical step toward understanding the adaptive value of various types of lunar cycles of reproduction.

Game theory and concepts such as evolutionarily stable strategy provide a helpful framework for thinking about the adaptive value of alternative phenotypes (Maynard Smith 1982, Gross 1996). Under this framework, a strategy is considered a genetically based decision rule that results in allocation of effort among alternative tactics; a tactic is a

phenotype; and the decision about which tactic is to be played is made by the strategy. Accordingly, phenotypic diversity may be categorized as 1 of 3 kinds of strategies: 'alternative', 'mixed' or 'conditional' (Gross 1996). Alternative strategies are genetic polymorphisms with single tactics, maintained by selection because the strategies have equal average fitness, e.g. some individuals always reproduce once per month while others always breed twice per month. In contrast, a mixed strategy is a genetic monomorphism with multiple tactics, maintained by selection because the tactics have equal average fitness, e.g. all individuals may reproduce once per month with one probability, or twice per month with another probability. A conditional strategy is also a genetic monomorphism with multiple tactics, but in this case maintained by selection because each tactic maximizes fitness when the individual is in a certain condition or context, e.g. individuals reproduce once per month when in poor condition or twice per month when in good condition.

We capitalized on a long-term field study and a carefully controlled laboratory experiment of individually identifiable clown anemonefish *Amphiprion percula* to investigate the individual-level strategies underlying population-level patterns of reproduction. In Madang Lagoon, Papua New Guinea, groups of *A. percula* are confined to magnificent sea anemones *Heteractis magnifica* that provide the fish with food, oviposition sites and protection from predators (Fautin 1992, Elliott & Mariscal 2001, Buston 2003a). Within each anemone there is a single group of *A. percula* composed of a breeding pair and up to 4 non-breeders that are excluded from breeding (Buston 2004a,b). Within each group, there is a size-based dominance hierarchy: the female is largest, the male is second largest, and non-breeders are progressively smaller (Buston 2003b, Buston & Cant 2006). *A. percula* is a protandrous hermaphrodite (Fricke & Fricke 1977, Moyer & Nakazano 1978): if the female of the group dies, then the male changes sex and assumes the position vacated by the female, and the largest non-breeder inherits the position vacated by the sex-changing male (Buston 2004a,b). Reproduction occurs throughout the year on a lunar cycle, and the breeders hatch hundreds of eggs each lunar month (Buston 2004a, Buston & Elith 2011).

Here, we investigated the individual strategies underlying the population-level lunar cycle in *A. percula*. We began by investigating whether all individuals breed once per lunar month or whether there is variation in the number of times that individuals breed each lunar month, i.e. whether there is a sin-

gle, pure reproductive strategy with a single reproductive tactic or whether there are alternative, mixed or conditional reproductive strategies (see Table 1) (Gross 1996). We proceeded to investigate whether the diversity of reproductive tactics, if it exists, represents alternative strategies, a mixed strategy or a conditional strategy (see Table 1) (Gross 1996). We used data from the long-term field study to discriminate a single strategy-tactic from alternative/mixed/conditional strategies and discriminate alternative strategies from mixed/conditional strategies; we used data from the laboratory experiment to discriminate mixed strategies from conditional strategies. We found that *A. percula* exhibits a conditional strategy, adjusting the number of times it breeds per lunar month to local food availability, which fundamentally alters inferences regarding the adaptive significance of population-level patterns of reproduction in this species and perhaps other marine species.

## MATERIALS AND METHODS

### Field context

We studied the clown anemonefish *Amphiprion percula* in Madang Lagoon, Papua New Guinea (5° 9' S, 145° 48' E), from January to December 1997 (Buston 2002). Madang Lagoon is bordered on its seaward edge by a submerged barrier reef and associated islands (Elliott & Mariscal 2001). Outside the lagoon, depths of 400 m are found close to the reef; inside the lagoon, the maximum depth is approximately 35 m. The outer barrier reef is very exposed during strong wind events; wave action is reduced on reefs within the lagoon. Water temperatures vary between 25 and 30°C throughout the year. Freshwater runoff can lower the salinity and increase the turbidity of nearshore lagoon sites after heavy rains. Fluctuations in water clarity, temperature and salinity are greater at nearshore sites than more offshore sites (Elliott & Mariscal 2001).

### Field population

Using SCUBA gear, we located 97 magnificent sea anemones *Heteractis magnifica* on 3 reefs (reef 1:  $n = 40$ ; reef 2:  $n = 31$ ; reef 3:  $n = 26$ ; Buston 2002). Each anemone was occupied by a single group of *A. percula*. Groups consisted of a breeding pair and 0 to 4 non-breeders (mean  $\pm$  SD number of individuals in each group:  $3.4 \pm 0.9$ ,  $n = 97$ ). We recognized individ-

uals  $\geq 18$  mm in standard length (SL) on the basis of natural variation in their color markings (Nelson et al. 1994, Buston 2003c), and we defined these individuals as residents (Buston 2003a). This study focused on the 71 groups found on reefs 1 and 2, which could be surveyed every other day; we did not include data from the 26 groups on reef 3, which could not be surveyed quite as regularly.

### Reproduction in the field

We monitored the reproductive activity of breeders in the field every 1 to 2 d for 10 lunar months. Breeding was readily detectable; in the days before spawning the male selected a nest site next to the base of the anemone and cleared it of debris, and in the days after spawning the male spent much of his time tending the eggs. The exact age of the eggs was determined on the basis of their color (day 1 = orange, day 2 = orange-brown, day 3 = brown, day 4 = black eyes, day 5 = silver eyes, day 6 = silver eyes with pupils, day 7 = gold eyes with pupils). The eggs hatched after 7 d.

### Lab context

We studied the clown anemonefish *A. percula* in the lab at Boston University from June 2014 to June 2015. We maintained sixty 114 l aquaria (30 gallon breeders) in 4 environmentally independent racks (15 aquaria rack<sup>-1</sup>). Each rack had its own continuous flow of recirculating saltwater (approximately 16700 l h<sup>-1</sup>; Instant Ocean Salt), its own pump (Reeflo Hammerhead), protein skimmer (My Creations Pro 2), and UV water treatment system. The abiotic conditions were kept as constant as possible: pH =  $8.30 \pm 0.34$ ; temperature =  $27.3 \pm 0.16^\circ\text{C}$ ; salinity =  $32.5 \pm 1.58$  ppt. The parameters were maintained 24 h d<sup>-1</sup>, 7 d wk<sup>-1</sup> by a Profilux computer controller. Manual testing for ammonia, nitrite and nitrate was done every week (Salifert and Red Sea test kits). Alkalinity was checked every few days using the Hanna alkalinity checker. Each aquarium was illuminated by 2 T5 24 W bulbs on a timer, giving the fish a 12 h day.

### Lab population

Each aquarium contained 1 cm of sand on the bottom, a 15 × 15 cm ceramic tile and a 10 × 10 cm reef rock—the rock and tile provided substrate for

anemones and fish breeding. Each tank housed 1 to 3 anemones *Entacmaea quadricolor* and a single pair of *A. percula*. All anemones were supplied by the Boston University Marine Program teaching laboratories, but their origin is unknown. Each tank was originally set up with 1 anemone, but the number of anemones per tank varied because the anemone population grew at a different rate in each tank, apparently influenced by the personality of the fish (Schmiege et al. 2017).

All fish originated from natural populations in Papua New Guinea and were supplied by Quality Marine and Sea Dwelling Creatures. Only fish <30 mm SL were acquired, ensuring that they were non-breeders in the wild. Removal of non-breeders is considered to be a sustainable practice because it has no negative effect on the number of breeding females, the per capita reproductive rate of breeding females or the overall population growth rate (Buston 2004a, Almany et al. 2017, Schlatter et al. unpubl. data). Upon arrival in our laboratory, we randomly paired each fish with one other fish and allowed them to establish dominance on their own (Wong et al. 2016). Pairs took  $\geq 2$  yr to start breeding (unpubl. data). This study focused on 20 of the 60 pairs that had bred together more than 10 times prior to the start of the study.

### Reproduction in the lab

We monitored the reproductive activity of the 20 pairs of breeders in the lab for 12 lunar months (Barbasch & Buston 2018). Pairs were fed a high ration of food (24 pellets pair<sup>-1</sup> d<sup>-1</sup>) for 6 lunar months, and a low ration of food (12 pellets pair<sup>-1</sup> d<sup>-1</sup>) for another 6 lunar months. Ten pairs received the high ration treatment first and the other 10 pairs received the low ration treatment first, enabling us to control for any effects of treatment order on behavior. As with

our observations of reproduction in the field, breeding in the lab was readily detectable by the male's behaviour. The age of the eggs was determined as above, and they likewise hatched after 7 d.

### Statistical analysis

First, we used field data to determine whether the *A. percula* population in Madang Lagoon exhibits a lunar cycle of reproduction, by generating a frequency distribution showing the number of clutches laid in the population on each lunar day. We tested the null hypothesis that reproduction is uniformly distributed throughout the month against the alternative hypothesis that reproduction is not uniformly distributed throughout the month, using a chi-squared test (Zar 2010). An alternative, Rayleigh's test, assumes that the population distribution does not have more than one mode, which we cannot assume (Zar 2010).

Second, we used field data to determine whether there is a single reproductive strategy/tactic (H0) or there are alternative (H1), mixed (H2) or conditional (H3) reproductive strategies (Table 1), by generating a frequency distribution showing variation among breeding pairs in the number of clutches laid per breeding pair per month. Third, we used field data to determine whether there are alternative strategies (H1) or whether there is a mixed (H2) or conditional (H3) strategy (Table 1), by generating a frequency distribution showing variation within breeding pairs in the number of clutches laid per breeding pair per month over the entire study period.

Fourth, we used lab data to determine whether there is a mixed (H2) or conditional (H3) strategy (Table 1), by conducting a generalized linear mixed effects model (GLMM) in which the number of clutches laid per lunar month is the dependent vari-

Table 1. Alternative hypotheses for reproductive strategies the clown anemonefish *Amphiprion percula* might possess, and discriminating predictions based on long-term field studies and a lab-based experimental manipulation of food availability. The existence of variation in reproductive tactics among individuals discriminates H0 from H1–H3; the existence of variation in reproductive tactics within individuals discriminates H0–H1 from H2–H3; the existence of variation in reproductive tactics associated with variation in state discriminates H0–H2 from H3. Taken together, tests of these predictions enable us to discriminate among all alternative hypotheses for the reproductive strategies that *A. percula* might possess

Test	H0: Pure reproductive strategies	H1: Alternative reproductive strategies	H2: Mixed reproductive strategy	H3: Conditional reproductive strategy
Variation in reproductive tactics among individuals (Fig. 2)	No	Yes	Yes	Yes
Variation in reproductive tactics within individuals (Fig. 3)	No	No	Yes	Yes
Variation in reproductive tactics associated with food (Fig. 4)	No	No	No	Yes

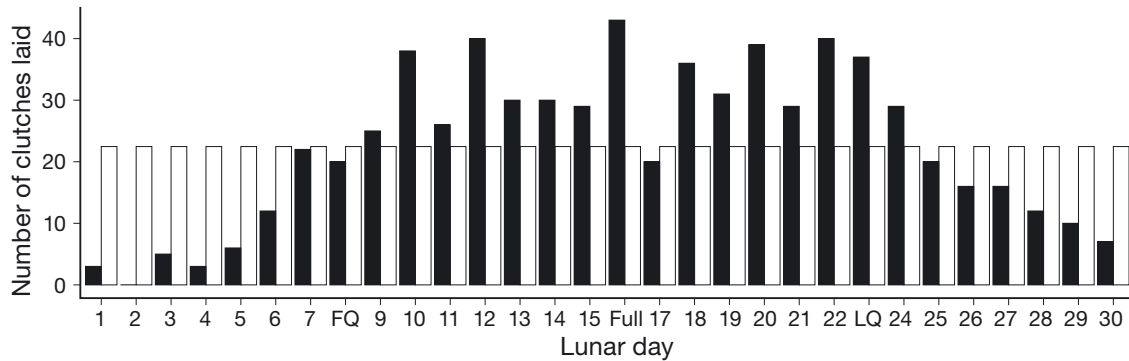


Fig. 1. Lunar cycle of reproduction at the scale of the *Amphiprion percula* population. Black bars: observed distribution of laying determined by field monitoring of 71 groups for 10 lunar months ( $n = 674$  clutches); white bars: expected distribution of laying determined by dividing total number of clutches by the number of days in a lunar month ( $n = 674$  clutches). FQ: first quarter; LQ: last quarter

able, and feeding treatment, treatment order and their interactions are the independent variables. Pair ID was considered a random effect, to control for the non-independence of repeated measures from the same pairs. We used a Poisson error distribution because the number of clutches laid is a discrete variable with integer values.

Finally, we used field data to investigate variation in the lunar cycle of reproduction for individuals laying 1, 2 and 3 times per lunar month, by generating a frequency distribution showing the number of clutches laid in the population on each lunar day by individuals expressing each of the 3 reproductive tactics. We tested the null hypothesis that reproduction is uniformly distributed throughout the month against the alternative hypothesis that reproduction is not uniformly distributed throughout the month using a chi-squared test for each of the reproductive tactics (Zar 2010). For individuals that bred 3 times  $\text{mo}^{-1}$ , bins of 2 d were used to ensure that the expected values were greater than 5. All statistics were conducted using R v.3.3.2 (R Core Development Team 2016).

## RESULTS

First, using field data, we investigated the lunar cycle of reproduction at the scale of the population in the clown anemonefish *Amphiprion percula*. The distribution of *A. percula* reproduction throughout the month showed a clear pattern which differed from that expected by chance (Fig. 1;  $\chi^2 = 215.14$ ,  $\text{df} = 29$ ,  $p < 0.001$ ). The most striking difference between the observed and expected distributions was that there was more reproduction than expected between first and last quarter of each lunar month and less repro-

duction than expected in the first and last 5 d of the lunar month (Fig. 1). Before we can infer the adaptive value of this pattern, we must determine what individuals are doing.

Second, using field data, we investigated whether there was variation in reproductive tactics among individuals of *A. percula* (Table 1, Fig. 2) by looking at the same data presented in Fig. 1 in a different way. The distribution of the number of clutches laid  $\text{pair}^{-1} \text{mo}^{-1}$  shows that there is variation in reproductive tactics among individuals, with individuals laying from 0 to 3 clutches  $\text{mo}^{-1}$  (Fig. 2): the mean ( $\pm \text{SD}$ ) number of clutches laid per month was  $1.2 \pm 0.94$ , the median was 1 and the mode was 2 (total number of clutches = 674; see also Fig. 1). This result falsifies the hypothesis that all individuals employed a pure reproductive strategy ( $H_0$ ; Table 1). However, we still cannot determine whether this variation represents alternative, mixed or conditional reproductive strategies ( $H_1$ ,  $H_2$ , or  $H_3$ ; Table 1). To determine this,

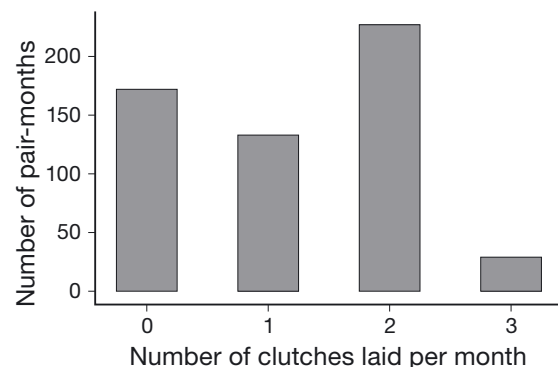


Fig. 2. Frequency distribution of the number of clutches laid per *Amphiprion percula* pair per month over the entire study period, revealing variation in reproductive tactics among individuals

we must determine whether there is variation in reproductive tactics within individuals.

Third, using field data, we investigated whether there was variation in reproductive tactics within individuals in *A. percula* (Table 1, Fig. 3). The distribution of the number of reproductive tactics used by individuals over the duration of the field study showed that there is variation in reproductive tactics within individuals (Fig. 3), with individuals expressing only 1 tactic (0, 1, 2 or 3 clutches  $\text{mo}^{-1}$ ), 2 tactics (0/1, 0/2, 0/3, 1/2, 1/3 or 2/3 clutches  $\text{mo}^{-1}$ ), 3 tactics (0/1/2, 0/1/3, 0/2/3, 1/2/3 clutches  $\text{mo}^{-1}$ ) or 4 tactics (0/1/2/3 clutches  $\text{mo}^{-1}$ ): the mean ( $\pm$ SD) number of tactics expressed was  $2.6 \pm 1.0$ , the median was 3 and the mode was 3. This result falsifies the hypothesis that individuals employed an alternative reproductive strategy (H1; Table 1). However, we still cannot determine whether this variation represents mixed or conditional reproductive strategies (H2 or H3; Table 1). To determine this, we must determine whether the variation in reproductive tactics was dependent upon conditions.

Fourth, using the laboratory experiment, we investigated whether the variation in reproductive tactics within and among individuals was caused by resource availability (Table 1, Fig. 4). Pairs laid significantly more clutches per month when fed a high ration of food than when fed a low ration of food, when controlling for the effect of treatment order and the interaction between feeding treatment and treatment order (GLMM;  $z = 0.196$ ,  $p = 0.00435$ ). This result falsifies the hypothesis that individuals employed a mixed reproductive strategy (H2), and provides support for the hypothesis that they employed a conditional reproductive strategy (H3; Table 1).

Finally, using field data once more, we investigated the variation in the lunar cycle of reproduction at the

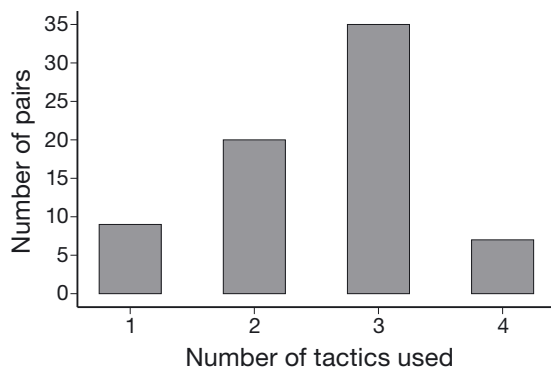


Fig. 3. Frequency distribution of the number of *Amphiprion percula* pairs expressing 1, 2, 3 or 4 reproductive tactics over the entire study period ( $n = 70$  pairs), revealing variation in reproductive tactics within individuals

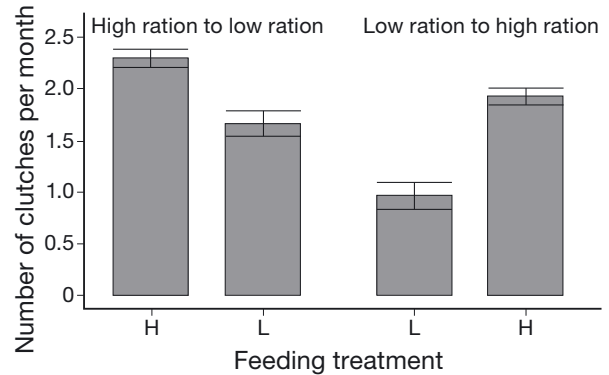


Fig. 4. Number of clutches laid by *Amphiprion percula* per lunar month, as a function of experimental manipulation of resource availability (food). Graph shows least squares mean estimates ( $\pm$ SE) of the number of clutches laid per month ( $n = 20$  pairs, 120 clutches); the 2 bars on the left show those fish that went from 6 mo on high food ration (H) to 6 mo on low food ration (L); 2 bars on the right show fish that went from 6 mo L to 6 mo H. In all cases, number of clutches includes only clutches laid in the second 3 mo (months 4 to 6) of each feeding treatment

scale of the population for individuals laying once, twice or 3 times per lunar month (Fig. 5). Considering individuals that laid once per month, there was a clear lunar cycle with a single peak in reproduction around the full moon and with no reproduction around the new moon. This pattern differed from that expected by chance ( $\chi^2 = 164.97$ ,  $df = 29$ ,  $p < 0.0001$ ). Considering individuals that laid twice per month, there was a clear semi-lunar cycle with 2 peaks in reproduction approximately 12 d apart. This pattern also differs from that expected by chance ( $\chi^2 = 220.93$ ,  $df = 29$ ,  $p < 0.0001$ ). Finally, considering individuals that laid 3 times per month, there was a clear trient-lunar cycle with 3 peaks in reproduction approximately 10 d apart. This pattern also differs from that expected by chance ( $\chi^2$ , based on 2 d bins = 57.66,  $df = 14$ ,  $p < 0.0001$ ). It is evident that individuals expressing different reproductive tactics follow different non-random lunar, semi-lunar ( $\frac{1}{2}$  lunar) or trient-lunar ( $\frac{1}{3}$  lunar) cycles of reproduction. Now that we know what individuals are doing, we can formulate clearer hypotheses regarding the adaptive value of the patterns.

## DISCUSSION

We found that there was a non-random, lunar cycle of reproduction at the population level in the clown anemonefish *Amphiprion percula*, consistent with observations of lunar and semi-lunar cycles of reproduction in other populations of *A. percula* (Madhu &

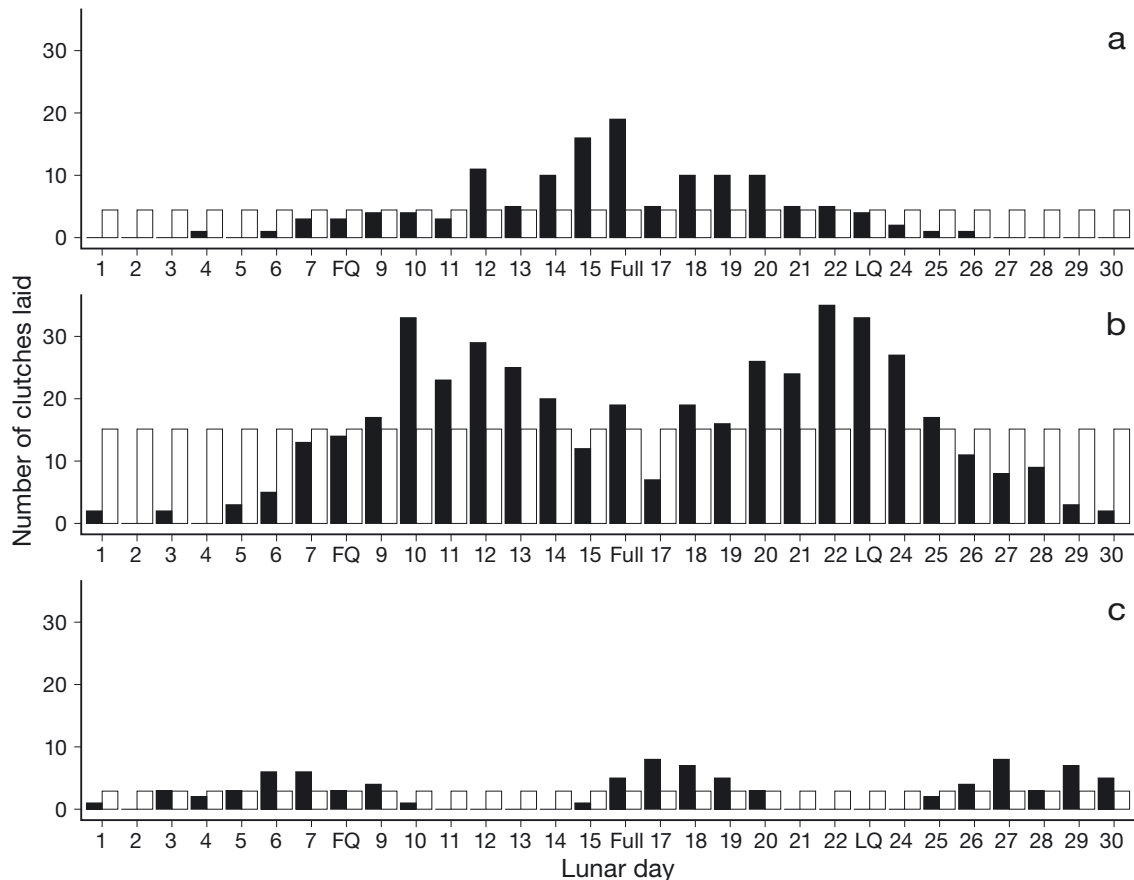


Fig. 5. Lunar cycles of reproduction at the scale of the *Amphiprion percula* population emerge from individuals employing a conditional strategy and expressing 1 of 3 reproductive tactics. Frequency distributions show the number of laying events per lunar day, split by tactic used in a given lunar month: individuals that breed (a) 1 time  $\text{mo}^{-1}$  (black bars,  $n = 133$  observed clutches; white bars,  $n = 133$  expected clutches); (b) 2 times  $\text{mo}^{-1}$  (black bars,  $n = 454$  observed clutches; white bars,  $n = 454$  expected clutches); and (c) 3 times  $\text{mo}^{-1}$  (black bars,  $n = 87$  observed clutches; white bars,  $n = 87$  expected clutches)

Madhu 2007), other anemonefishes (Allen 1972, Ross 1978, Richardson et al. 1997) and other coral reef fishes (Johannes 1978, Robertson 1991). Deeper investigation revealed that this population-level pattern is an emergent property of variation in reproductive tactics among and within individuals—individuals breeding once, twice or 3 times a month. Further examination revealed that individuals employed a conditional strategy, with the number of times they breed each month dependent on environmental conditions. Breaking down the population-level pattern by reproductive tactic, we showed that each reproductive tactic had its own non-random lunar, semi-lunar or trient-lunar cycle of reproduction.

With these patterns identified, we can start to think more rigorously about the adaptive value of reproductive timing, but we must first consider the effect of food availability and other factors on the frequency of reproduction. We have shown that *A. percula* lays

more clutches per month when fed a high food ration than when fed a low food ration. Effects of food availability, temperature and seasonality on the frequency of reproduction have been documented in other studies of *A. percula* (Madhu & Madhu 2007, Dhaneesh et al. 2012), other anemonefishes (Allen 1972, Richardson et al. 1997) and other fishes (Izquierdo et al. 2001, El-Sayed et al. 2003). Thus, documenting an effect of food availability on the frequency of reproduction is not novel. Here, the novelty lies in recognizing the implications of variation in frequency of reproduction for our inferences regarding the adaptive value of reproductive timing.

The adaptive value of reproductive timing should not be inferred based on the population-level lunar cycle, because this population-level pattern is an emergent property that comes from pooling multiple types of individuals. Rather, the adaptive value of the lunar, semi-lunar and trient-lunar cycles should be

investigated for the individuals that express them. Recognizing this, the appropriate questions to ask are (1) why do individuals that exhibit a lunar cycle of reproduction reproduce around the full moon, (2) why do individuals that exhibit a semi-lunar cycle of reproduction reproduce just after the first quarter and just before the third quarter and (3) why do individuals that exhibit a trient-lunar cycle of reproduction reproduce just before the first quarter, around the full moon and just after the third quarter? In each case, the timing is clearly non-random and alternative timings are possible but not chosen. Whether there is a net benefit to adults, eggs, larvae or settlers of reproducing at a particular time or avoiding reproducing at other times cannot be inferred from the patterns of reproduction alone, but now, at least, it is clear which hypotheses should be tested.

Rigorously testing the alternative hypotheses concerning the adaptive value of the lunar, semi-lunar and trient-lunar cycles will be a formidable challenge. One would have to investigate whether there is an association between lunar day of reproduction and the number of eggs laid (adult biology hypothesis), lunar day and the proportion of eggs hatched (egg biology hypothesis), lunar day and the probability of successful dispersal (larval biology hypothesis) and lunar day and the probability of successful recruitment (settler biology hypothesis), capitalizing on the small amount of natural variation in the timing of the events. This would have to be done separately for the individuals that reproduce on the lunar, semi-lunar and trient-lunar cycle. *Amphiprion* is perhaps the one genus where this might be possible because entire populations can be monitored, the number of eggs laid and hatched can be measured (Buston 2004a, Buston & Elith 2011, Saenz-Agudelo et al. 2015), the number of settlers and recruits produced can be measured (Buston 2003a, Saenz-Agudelo et al. 2015, Almany et al. 2017), as can the timing of their production via daily monitoring (this study) and otolith analyses (Berumen et al. 2010).

In summary, in clownfish, and likely many other coral reef fishes, lunar cycles of reproduction observed at the population level can be an emergent product of lunar, semi-lunar and trient-lunar cycles of reproduction at the individual level. In clownfish, and likely many other coral reef fishes, individuals employ a conditional strategy, breeding once, twice or 3 times a month depending on local food availability. Likely, individuals that are in different condition or contexts will face different reproductive tradeoffs, and different combinations of hypotheses with different weights will explain the adaptive value of the dif-

ferent reproductive cycles. The important message is that the adaptive value of lunar cycles of reproduction should not be inferred from or hypothesized based on the population-level pattern if there are multiple types of individuals within the population employing different reproductive cycles. In these cases, the adaptive value of the lunar, semi-lunar and trient-lunar reproductive cycles should be investigated for the individuals that express them.

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